In tests at the Beltsville laboratory, a vaccine injection is used to immunize chicks against coccidia with recombinant DNA containing two promising proteins from the outside coat of oocysts, the protozoa's infectious stage.

KEITH WELLER (K8277-5)

Making Coccidia Less Cocky

Each year, U.S. poultry producers raise about 7 billion broilers. That's a lot of beaks to feed. And feed is the costliest item on the producer's balance sheet—topping even labor costs.

That's why chickens are routinely given drugs to prevent infection by tiny, single-celled protozoa known as coccidia. These organisms invade cells in a chicken's gut, where they reproduce and make it harder for the bird to absorb feed and gain weight quickly.

Coccidiosis is among the top-five chicken diseases that prevent weight gain, says Agricultural Research Service microbiologist Harry D. Danforth. He is with ARS' Parasite Biology and Epidemiology Laboratory at Beltsville, Maryland. Each year, the protozoa cost producers worldwide an estimated $600 million in treatment and low carcass weights.

And it could become worse, because the protozoa are developing resistance to standard drugs. That has Hyun S. Lillehoj and Mark C. Jenkins, who are based at the ARS Immunology and Disease Resistance Laboratory at Beltsville, working on ways to use the birds' own immunity against coccidia. "We have short- and long-term goals," says Danforth, who is the agency's scientific liaison with the poultry industry. The short-term goal is a gamma-irradiated vaccine Danforth tested this year at Perdue Farms, Inc., in Salisbury, Maryland.

First, however, Jenkins had to determine the radiation dose needed to weaken the live oocysts—the infectious stage of coccidia. This prevents them from developing or reproducing. Next, he figured the dose of weakened oocysts needed to produce immunity in the chicks. Then, Danforth put the oocysts in a gel delivery system he and researchers in the vaccine industry had developed earlier to get live vaccine into chicks at the hatchery. The gel was added to the feed of chicks destined to become Cornish hens.

Danforth says it takes about 1.6 pounds of feed for each pound of bird. With the gamma-irradiated oocysts, "feed conversion was 3 points better than with the anticoccidial drugs," he says. "That means the treatment reduced, by three hundredths of a pound, the feed needed to raise a 2-pound Cornish hen. That may seem small, but it's nothing to sneeze at when multiplied by thousands or millions of birds. The company would realize an extra $1 million a year for its Cornish hens alone, says Danforth.

Donna Hill, Perdue's director of health services, says the vaccine had other benefits. The birds were more uniform in size and had good color. The results were so promising that "if it were commercially available, we would probably start using an attenuated vaccine right away for Cornish hens and begin testing it on broilers," she says.

Trouble is, it takes 10,000 of the killed oocysts to immunize each bird, and the oocysts have to be grown in live chickens. About 40 billion gamma-irradiated oocysts per week would be needed just to inoculate all the broilers raised by Perdue alone, says Danforth. "It's a numbers game. We're trying to give the producer some relief now, until researchers streamline ways to enhance chickens' immune response to coccidia."

And that's no easy task.

Not Too Little—Not Too Much

The chicken's immune system is more complicated than a Chinese puzzle. And launching an immune response is walking a fine line. It can protect a bird—or destroy it, if it goes too far. So researchers first have to unravel the complex inner workings in the bird's gut before they can get the optimum immune response without an overreaction.

Jenkins approached this problem by identifying proteins in the oocysts that mark it as an intruder and elicit an immune response. He, Lillehoj, Danforth, and Michael D. Ruff have a patent on the recombinant DNA for two promising proteins from the oocysts' outside coat.

Jenkins inserted the recombinant DNA for those two coat proteins and another promising protein separately into DNA loops, called plasmids, taken from *E. coli* bacteria. In small-scale tests at the Beltsville lab, he shot the plasmids straight into chicken legs using a jet gun, like the ones dentists use to numb their patients' teeth and gums.

"We got the best protection with mixtures of all three plasmids," Jenkins says. Weight gain in the immunized chicks was significantly better than in the unimmunized birds. But it was not as good as in the birds that never got close to a coccidia oocyst. "We have a little way to go because we want to have complete protection," he says. And a more efficient system for delivering such an inoculum in an industry setting is needed.

Lillehoj's lab identified another promising protein. It enables the stage that emerges from the oocyst, called a sporozoite, to invade the bird's T cells. Lillehoj patented a monoclonal antibody to the protein that she says "consistently blocks this invasion in culture dishes." She is now collaborating with scientists in Japan and Korea to find the DNA that directs production of that protein.

The All-Natural Boost

Lillehoj's main focus is on the substances immune cells generate to communicate with one another. Animals produce these natural, hormonelike chemicals, called cytokines, during an infection. They are potent and function at low levels.

Some cytokines enhance the immune response. But others can cause disease symptoms, "so you have to know which ones are protective," says Lillehoj. "Understanding how this works may be a way to control infection without introducing anything unnatural."

The chicken's immune system is more complicated than a Chinese puzzle.

She is looking for umbrella protection against the six or seven chicken coccidia species because, once cytokines are produced, they aren't picky about the species. They may also be given along with vaccines to increase their effectiveness. The challenge is that there are more than 20 different cytokines that regulate immune response, Lillehoj says. "We're just beginning to understand how they work."

One all-purpose cytokine that has proved effective is interferon gamma. (See "Two Strategies for Protecting Poultry From Coccidia," *Agricultural Research*, Oct. 1996, pp. 12-13.) Interferon gamma activates macrophages—cells that behave like the Pac Men of the immune system, gobbling up invaders. Interferon gamma inhibits coccidia multiplication, so the birds lose less weight. Lillehoj says the birds' immunity level correlates with their interferon gamma level.

Another cytokine that's showing promise in laboratory tests is interleukin-15. IL-15 prompts the all-important, infection-fighting T cells to multiply. Last year, Lillehoj's lab cloned the gene for IL-15, and they are testing its use under three protocols.

In one, they inject the IL-15 protein the gene produces directly into the chick's muscle. In another, they use a gene gun to inject the chick with the naked IL-15 DNA. And in the third, they insert the DNA into a vector, such as a weakened version of the fowl pox virus that is now used to immunize poultry against fowl pox.

"All three methods enhance the chickens' innate immune response against coccidia," Lillehoj says. "The animals have more T cells, which are critical for defense, and they lose less body weight."

Lillehoj is collaborating with three companies on chicken cytokine research. But progress has been slow, she says, because knowledge about human cytokine DNA isn't much help. Poultry DNA is only about 30 percent similar to human DNA, compared to a 70-percent similarity in large meat animals.

Lillehoj is also collaborating with Perdue on a different tactic for controlling coccidiosis. Since some chickens are genetically more resistant to the disease than others, her lab is searching for the genes that confer this resistance. Once they are identified, poultry producers can breed for the hardiest birds.—By **Judy McBride,** ARS.

Harry D. Danforth is at the USDA-ARS Parasite Biology and Epidemiology Laboratory, 10300 Baltimore Ave., Beltsville, MD 20705-2350; phone (301) 504-8300, fax (301) 504-5306, e-mail hdanfort@lpsi.barc.usda.gov.

Mark C. Jenkins and Hyun S. Lillehoj are at the USDA-ARS Immunology and Disease Resistance Laboratory, 10300 Baltimore Ave., Beltsville, MD 20705-2350; phone (301) 504-8201, fax (301) 504-5306, e-mail mjenkins@lpsi.barc.usda.gov hlilleho@lpsi.barc.usda.gov. ◆